PE Screening Test for Blood

A. SCOPE

A.1 When a suspected bloodstain is submitted to the laboratory, it is typically visually examined first and then subjected to a quick, sensitive, but non-specific presumptive test to determine if it could be blood. This presumptive test relies upon the catalytic peroxidase-like activity of the heme group of hemoglobin. Therefore, this test will react with blood from animals as well as humans. Hemoglobin and a number of its derivatives catalyze the oxidation by peroxide of a number of organic compounds to yield colored products. Presumptive testing for the presence of blood will be performed in the laboratory typically utilizing the Kastle-Meyer test.

B. QUALITY CONTROL

B.1 Known positive and negative controls must be tested when a new phenolphthalein stock solution is prepared.

B.2 Results must be documented in the Laboratory Assets Management System (LAM).

B.3 A bloodstain control must be tested each day of use prior to testing unknown or suspected blood samples from casework.

B.4 Results of the day of use quality control testing must be documented in the case notes and include the lot#, expiration date and the control utilized.

B.5 If the used quality control measures do not produce the expected result, the test will not be utilized on evidentiary samples and troubleshooting will be performed. New solutions or materials may be required.

C. SAFETY

C.1 Treat all biological samples as potentially infectious. Gloves, a face mask, eye protection (e.g. safety glasses or a face shield) and a lab coat must be worn.

C.2 All appropriate SDS sheets must be read prior to performing this procedure for the first time.

C.3 Distinguish all waste as general, biohazard or sharps and discard appropriately.

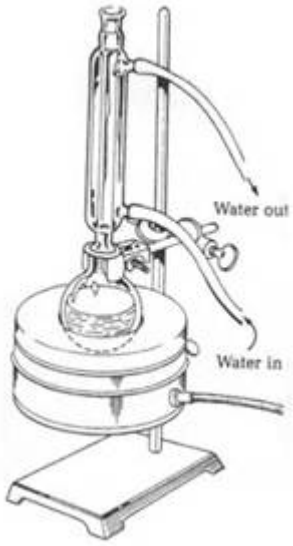
C.4 The zinc granules utilized to layer the bottom of the phenolphthalein stock solution bottle and the Solution 1 bottle must be air dried prior to discarding. Remove from the respective bottles, place zinc granules in a glass petri dish in a fume hood and air dry completely. Once dry, place zinc granules in a plastic bag and discard.

C.5 Should the Solution 1 bottle become cloudy during use, a rinse of the empty otherwise clean bottle with a small amount of concentrated acetic acid in a fume hood may be performed.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 Make the phenolphthalein stock solution

D.1.1 Using cold water and an apparatus attached to a retort stand, reflux 2.0 g phenolphthalein, 20 g potassium hydroxide, and 100 ml distilled water with 20 g powdered zinc and boiling chips in a fume hood until the solution becomes colorless (0.5-2 hrs).



D.1.2 Store this solution in a dark bottle with a layer of zinc granules to keep it in the reduced form. Assign lot# and expiration date (date of earliest expiring component) and document this in the Laboratory Assets Management System (LAM). Discard should the solution become noticeably pink prior to the assigned expiration date.

D.2 Make Solution 1

D.2.1 Prepare a 1:5 dilution of the phenolphthalein stock solution by mixing 5 ml of phenolphthalein stock solution with 20 ml of absolute ethanol.

D.2.2 Store with a layer of zinc granules and discard if it becomes noticeably pink.

D.3 Make Solution 2

D.3.1 Prepare a 1:10 dilution of stock 30% hydrogen peroxide by mixing 2 ml of 30% hydrogen peroxide with 18 ml of deionized water.

D.3.2 For the combination of solution 1 and solution 2, assign a lot# and expiration date (date of earliest expiring component) and document this in the Laboratory Assets Management System (LAM).

D.4 A known bloodstain is used to test the solutions.

E. EQUIPMENT

E.1 Reflux apparatus

E.2 Boiling chips

E.3 Dark bottle

E.4 Storage bottles with droppers

E.5 Filter paper or cotton swab

E.6 Scissors

F. PROCEDURES

F.1 Scratch the suspected stain with the point of a folded filter paper sufficiently to transfer color onto the filter paper. Alternatively, moisten a cotton swab with deionized water and hold in contact with the stain long enough to transfer color or remove a very small cutting (~1-2 mm^2) if color transfer is not observed. Additional

options for certain situations may include a slightly moistened filter paper (i.e. a minute stain that you do not wish to disturb or consume).

F.2 Unfold the filter paper and add one drop of Solution 1 at the point of colour transfer allowing it to absorb into the filter paper. Alternatively, add one drop of Solution 1 to the moistened cotton swab or cutting.

F.3 Add one drop of Solution 2 at the point of colour transfer to the filter paper, swab or cutting.

G. INTERPRETATION GUIDELINES

G.1 If a bright pink (fuchsia) color develops almost immediately after the addition of Solution 2, this indicates a positive result for the presumptive presence of blood. Weaker stains may exhibit slightly delayed results and the color will be less intense.

G.2 No color change after 30-60 seconds indicates a negative result.

G.3 Limitations

G.3.1 Color development before the addition of Solution 2 may be due to the presence of a chemical oxidant.

G.3.2 Several substances may give a positive color reaction. Copper and nickel salts, rust, and vegetable peroxidases will frequently show positive color reactions. Plant peroxidases react similarly to blood in catalyzing this reaction. However, they are generally associated with plant tissue and can be visually distinguished from blood. In addition, they tend to be unstable over time, losing their ability to oxidize the phenolphthalein reagent. Many substances of animal origin may contain blood (even in trace amounts) and will therefore give a positive result.

H. REFERENCES

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